Bemisia argentifolii (Homoptera: Aleyrodidae) Colonization on Upland Cottons and Relationships to Leaf Morphology and Leaf Age

CHANG-CHI CHU,[1] THOMAS P. FREEMAN,[2] JAMES S. BUCKNER,[3] THOMAS J. HENNEBERRY,[1] DENNIS R. NELSON,[3] GREGORY P. WALKER,[4] AND ERIC T. NATWICK[5]

Western Cotton Research Laboratory, USDA–ARS, 4135 E. Broadway Road, Phoenix, AZ 85040

Ann. Entomol. Soc. Am. 93(4): 912–919 (2000)

ABSTRACT We compared *Bemisia argentifolii* Bellows & Perring colonization on Stoneville (ST) 474 and Deltapine (DPL) 5415 cottons, *Gossypium hirsutum* L., in the field. We also examined leaf trichome density, leaf age, and leaf morphological characteristics as possible factors influencing cultivar host selection. The increased numbers of all *B. argentifolii* life stages on ST 474 in the field appeared to be related to the higher trichome density on abaxial leaf surfaces compared with DPL 5415. In both cultivars, leaves from node number 1 below the terminals were smaller and had higher vascular bundle densities and numbers of lysigenous glands than older, larger leaves. Younger leaves also had smaller leaf areole areas, more terminal vein endings per unit leaf area, and shorter distances from abaxial leaf surfaces to minor vein phloem tissues compared with older leaves. These younger leaf morphological characteristics may contribute to the higher *B. argentifolii* densities on younger leaves. In the laboratory, electronically monitored adult females and visually monitored settled first and fourth instars preferred to probe into secondary and tertiary leaf veins as compared with main and primary leaf veins.

KEY WORDS *Bemisia tabaci* strain B, *Bemisia argentifolii*, leaf age, vascular bundles, trichomes, vein endings

DESPITE EXTENSIVE USE of insecticides for control, cotton losses caused by *Bemisia argentifolii* Bellows & Perring in Arizona and California in 1997 were estimated at 21,059 bales (William 1998). A long-term whitefly management solution that is both economical and environmentally acceptable is needed. In the United States, differences in cotton plant susceptibility to colonization by *B. argentifolii*, also referred to as *B. tabaci* (Gennadius) strain B, have been reported between upland, *Gossypium hirsutum* L., and Pima, *G. barbadense* L., cottons (Natwick et al. 1995, Percy et al. 1997) and among different cultivars of upland cottons (Chu et al. 1998a, 1999a). These differences have been attributed to variations in leaf pubescence, i.e., genotypes with more trichomes are generally more susceptible than genotypes with smooth leaf characteristics (Butler and Henneberry 1984, Flint and Parks 1990, Butler et al. 1991, Norman and Sparks 1997, Percy et al. 1997), and to differences in leaf shapes, i.e., okra-leaf genotypes are generally more resistant than normal-leaf genotypes (Berlinger 1986, Chu et al. 1999a).

The development of insect resistant cultivars by conventional plant breeding and selection are approaches that warrant increased attention for insect population suppression (Sippell et al. 1987). Where successful host plant resistance has been developed, the effect on insect populations has been dramatic, and the methodology is readily accepted as economically sound (Jenkins 1994). We have conducted a number of studies to identify mechanisms of cotton plant resistance to *B. argentifolii* infestations. Our objectives are to identify genetic plant traits that may be incorporated by plant breeders into acceptable agronomic cotton backgrounds. In this study, we compared *B. argentifolii* colonization on Stoneville (ST) 474 and Deltapine (DPL) 5415 cottons in the field. We examined trichome density, leaf morphology, and leaf age as possible factors in host selection. The leaf morphology traits we examined were sizes and numbers of leaf areole areas, numbers of lysigenous glands, numbers of leaf terminal vein endings, leaf thickness, and distance from abaxial leaf surfaces to the nearest minor vein phloem tissues. Additionally, in the laboratory, we visually and electronically monitored *B. argentifolii* adult and visually monitored nymph stylet probing site selection.

Materials and Methods

Field and Greenhouse *B. argentifolii* Density, Cotton Leaf Age, and Morphological Characteristics. *B. argentifolii and Leaf Trichome Densities.* Upland cotton

[1] Western Cotton Research Laboratory, USDA–ARS, Phoenix, AZ 85040.
[2] Electron Microscopy Center, North Dakota State University, Fargo, ND 58105.
[3] Biosciences Research Laboratory, USDA–ARS, Fargo, ND 58105.
[4] Department of Entomology, University of California, Riverside, CA 92521.
[5] Imperial County Cooperative Research and Extension Center, University of California, Holtville, CA 92250.

seeds were planted in the field on 28 April 1998 at the University of Arizona Maricopa Agriculture Research Center, Maricopa, AZ. The cotton seeds were treated with a mixture of insecticides and fungicides before they were shipped from seed companies. The experimental design was a randomized complete block with four replicates. Plots were 12.2 by 8 m, with 1 m between rows. Treatments were two cultivars, ST 474 and DPL 5415. Oxamyl (DuPont, Wilmington, DE) was applied on 25 June to control plant bugs, *Lygus hesperus* Knight. No other pesticides were applied during the growing season. Weeds were removed with discs mounted on a tractor before the close of plant canopy in August. Plants were irrigated as needed, about once every 10–20 d, depending on growth of plants. *B. argentifolii* adults were counted on fifth main stem node leaves below each terminal on each of 10 randomly selected plants in each plot (Naranjo and Flint 1995). Also, fifth main stem node leaves were picked from each of 10 additional plants in each plot for counting numbers of eggs and nymphs. Egg and nymph counts on abaxial surface of leaves were made with the aid of a stereoscope (magnification of 10–50×) on one 3.8-cm^2 leaf disk taken at the base of the second sector of each sampled leaf (Naranjo and Flint 1994). There were 10 sample dates at weekly intervals from 22 July to 30 September. In conjunction with these studies, we grew ST 474 and DPL 5415 plants in the greenhouse to determine the number of single-cell, nonglandular trichomes on the fifth node leaves of each cultivar.

Leaf Age. We randomly chose one plant of ST 474 and of DPL 5415 from each of three replicated cotton plots ($n = 3$ plants per cultivar) to study morphological characteristics of different age leaves. At dawn of 6 August, leaves on main stems with attached petioles were picked at nodes #1, 3, 5, 7, 10, 15, and 20 numbered from top to bottom of the plants. Leaves at node #1 were ≥2.5 cm between the two largest leaf lobes, and leaves at node #20 were the bottom leaves on main stems. Petioles of picked leaves were placed in water-filled plastic floral tubes packed with blue ice and shipped overnight to the North Dakota State University Electron Microscopy Center, Fargo, ND.

Leaf segments (1 cm^2) were randomly selected from the second leaf sector of three leaves (Naranjo and Flint 1994) from each leaf node for each cultivar. Each leaf segment was cut into 1-mm^2 pieces, fixed in 0.25 M glutaraldehyde in 0.1 M Milloig's phosphate buffer (pH 7.4) for 3 h at room temperature (23°C). The samples were washed twice with buffer and post-fixed in 7.8×10^{-2} M osmium tetroxide in buffer at 4°C, for 4 h. Following another wash in buffer, the samples were dehydrated at room temperature (23°C) in a graded series of acetone (30, 50, 70%-saturated with uranyl acetate, 90%, 100% [two times]) for 30 min each with the exception of 4 h for the 70% acetone-uranyl acetate step. Following dehydration, the samples were infiltrated and embedded in Epon-Araldite blocks. Thin sections (1–2 μm) were cut from the blocks with a RMC ultramicrotome (RMC, Venana Medical Systems, Tucson, AZ). Sections were placed on glass slides and stained with toluidine blue O, examined, and photographed. Measurements of leaf thickness and distances between abaxial leaf surface and nearest minor vein phloem tissues were measured with a Leitz compound light microscope equipped with an filar micrometer eyepiece. Eight measurements each from different phloem tissue were made on each of three replicated leaves from each of seven leaf nodes for each cultivar.

Leaf and Areole Areas. Tracings of the fresh leaves (three replicates from the seven leaf nodes) were made and the area of each leaf was calculated. Photographs of the abaxial leaf surfaces were obtained at 50× magnification using an Olympus SZH dissecting microscope. The images were recorded on Kodak Elitechrome slide film (ASA 200).

Photographic images of the cleared, 1-cm^2 leaf segments were made using an image scanner and imported into a computer using Adobe Photoshop (version 5.0, Adobe System, San Jose, CA) and TWAIN-32 cross-platform interface, and saved as tagged-image file format files. An image analysis program (OPTIMAS, Media Cybernetics, Silver Spring, MD) was used to calibrate, measure, and format leaf areole area data. Leaf areole areas were determined from 37-mm^2 leaf areas from each of two photographic images for leaves from nodes #1 and #15 for each cultivar. Numbers of areoles measured were 315 and 174 for leaf node leaves #1 and #15, respectively, for ST 474, and 267 and 165 for leaf node leaves #1 and #15, respectively, for DPL 5415. Vein areas, including bundle sheath extensions (McClendon 1992) on each of three 0.75-mm^2 leaf areas were outlined around and in areoles on leaf node #7 with a mouse on a computer screen and the surface areas were calculated. Percentages of vein areas were calculated from the total leaf areas.

Leaf Terminal Vein Endings. Additional leaf segments of 1 cm^2 each from the basal portion of the same three leaves (at 5 mm distance from the tip of the petiole and 10 mm left and right of the center vein) for each node of each cultivar were placed in clearing agents. For leaf nodes #1, 3, and 5, 95% ethyl alcohol solution was used to clear leaf segments at room temperature (23°C) for a minimum of 30 min. For leaf nodes #7, 10, 15, and 20, chloroform-methanol (1:1) was used to clear leaf segments as ethyl alcohol alone did not provide an adequate clearing. The time for clearing older tissues ranged from 1 h at room temperature to several weeks, and boiling temperatures were frequently used. Cleared leaf segments were stained in aqueous 1% safranin O and destained as necessary in acidified ethyl alcohol. Stained leaf segments were mounted on glass slides, examined and photographed using an Olympus BH-2 compound microscope. Numbers of terminal vein endings were counted in 4–15 leaf segments for each of three replicates of the five leaves from nodes #1, 7, 10, 15, and 20 of each cultivar.

Lysigenous Glands. Two additional photographs were taken of the images (three replicates) of leaves from nodes #1, 3, 5, 7, 10, 15, and 20 from both ST 474 and DPL 5415 cultivars at the basal and tip portions of

each leaf. Photographs at leaf base were at 5 mm distance from the tip of the petiole and 10 mm left and right of the center main vein. The photographs near the leaf tip were taken at 10 mm left and right of the center vein at the edge of the leaf lobes. Photographs were taken with Kodak Elitechrome film (ASA 200) at 50× magnification using an Olympus SZH dissecting light microscope. The numbers of lysigenous glands per unit area were counted.

Laboratory Studies—Leaf Probing Sites of Adults and Settled First and Fourth Instars. For defining the locations of *B. argentifolii* adult female and nymph stylet probing sites, we classified the cotton leaf vein complex into main, primary, secondary, and tertiary veins. Main veins were the five veins that extended from the leaf base and petiole juncture to the leaf edges. Veins branching from main veins were classified as primary veins and subsequently secondary and tertiary as veins branching from primary and secondary veins, respectively. Tertiary veins surrounded the leaf areole areas. Areole areas have lysigenous glands located at their centers. Lysigenous glands contain secondary products including gossypol (Bell 1986). Tertiary veins can be positively identified under a stereoscope (magnification of 10–50×), but the veins that branch from them cannot be distinguished except with more sophisticated high magnification microscopy.

Stylet probing sites of adults on abaxial leaf surfaces were determined by direct observation and electrical recording of stylet penetration graphs (EPG) (Missouri Electronic Insect Feeding Monitor Type 2.1, Electronic Instrument Laboratory, University of Missouri, Columbus, MO). The EPG was connected to a Hewlett–Packard computer equipped with a data acquisition program (WinDaq/Pro and WinDaq/Pro+, Dataq Instrument, Akron, OH). Adult females were immobilized on a cold plate at 14.1°C. They were tethered on 2.5-μm-diameter platinum wires (Wollaston process wire, Sigmund Cohn, Mt. Vernon, New York, NY) attached to dorsal thorax integuments with silver paint (Walker and Janssen 2000). Tethered females were acclimated overnight and placed on second leaf sectors of abaxial leaf surfaces. Locations were ≈1.5 cm from the leaf base and between the two main veins. Leaves were those on the fourth or fifth main stem leaf node from terminals of greenhouse grown DPL 5415 cotton plants. Abaxial leaf surfaces were tilted at 55° angle under 38.9 Klux (= 291 W m^{-2}) light intensity to observe stylet penetration activity. The stylet probing sites, on specific veins or between veins, were recorded. Stylet penetration was confirmed when a series of sawtooth electrical waveforms (Walker and Perring 1994) occurred on the computer screen. The probing activities of ten adult females were monitored for 2 h using three leaves from three plants with four females on one leaf and three females on each of two other leaves.

The stylet probing sites, on specific veins or between veins, of settled first and fourth instars on abaxial leaf surface of DPL 5415 were determined on 10 randomly selected leaf areas (39 mm^2 each) on five leaves from the fourth or fifth main stem nodes as previously described for adults. One settled first and one fourth instar was randomly chosen in each area to examine for leaf penetration sites with the aid of a stereoscope. We also measured the body lengths of adult males and females (100 each) and settled first and fourth instars (100 each). The objective was to consider body length in relation to access to the feeding sites and phloem tissue sources.

Data Analyses. Numbers of *B. argentifolii* adults per leaf-turn, eggs and nymphs per square centimeter of leaf disk were plotted against weekly sampling dates for each of the two cultivars to show the changes in population densities during the season. The counts for the 10 weekly samples of adults, eggs, and nymphs were averaged for each plot and transformed using square root plus one when test results indicated heterogeneous variance. Means were analyzed using analysis of variance (ANOVA) (Anonymous 1989) for a randomized complete block design. Data transformation using square root plus one, were also done for other data measurements when necessary. All other data, except where noted, were analyzed using ANOVA for appropriate experimental designs. Data for *B. argentifolii* adults and settled first instar body lengths were averaged and standard errors of the means were calculated. Statistical significance for differences for adult and settled first and fourth instar probing sites was analyzed using chi-square analysis.

Means for each data set, where appropriate, were separated using Student-Neuman-Keul's multiple range test at $P = 0.05$, following a significant F test. A regression analysis was made to determine the relationship between the phloem tissue depth and leaf thickness.

Results

Field and Greenhouse *B. argentifolii* Density, Cotton Leaf Age, and Morphological Characteristics. *B. argentifolii and Leaf Trichome Densities.* Weekly mean numbers of adults per leaf and eggs and nymphs per square centimeter were greater, in most cases, on ST 474 than on DPL 5415 (Fig. 1 A–C). Seasonal mean numbers, over all sampling dates, of adults, eggs and nymphs were 2.6, 17 and 10 times higher for ST 474 than DPL 5415 (Table 1). ST 474 had 20 times more trichomes on fifth node leaves than DPL 5415 (Table 2). Most ST 474 trichomes were on primary veins followed by secondary and tertiary veins, and between veins. A few trichomes were found on DPL 5415 primary veins but few or no trichomes at other leaf locations.

Leaf Age. Over all leaf ages, the mean distances from abaxial leaf surfaces to phloem tissue between the two cultivars were not significantly different (Table 3). On average for both cultivars, leaves from node #1 had the shortest distances from abaxial leaf surface to phloem tissues compared with all other older leaves. The distances for both cultivars tended to increase gradually with leaves from node #3 to node #10 leaves but differences were not significant except for node

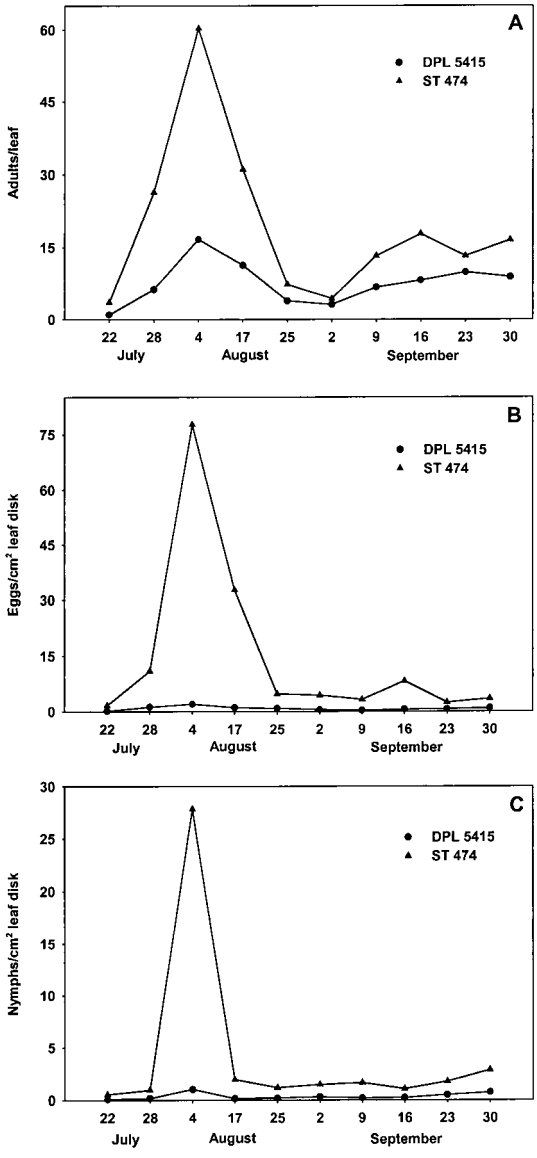

Fig. 1. Mean numbers of *B. argentifolii* on ST 474 and DPL 5415 (A) Adults/cotton leaf-turn. (B) Eggs per square centimeter on leaf disks. (C) Nymphs per square centimeter on leaf disks, in each case, from 22 July to 30 September 1998 at Maricopa, AZ.

#15 and #20 leaves compared with leaves from nodes #1 or #3.

On average, DPL 5415 leaf thickness was greater than ST 474. In general, leaf thickness increased with leaf ages. On average for both cultivars, leaves from node #1 and node #20 were the thinnest and the thickest, respectively. Leaves from nodes # 1, 7, and 10 of DPL 5415 were thicker than ST 474 leaves from the same nodes. Regression analysis of the relationship between depth of phloem tissue and leaf thickness showed that the two cultivars were significantly different (Fig. 2). The depth of phloem tissue in relation to leaf thickness was best described as a quadratic function for ST 474, but a linear function for DPL 5415.

Table 1. Seasonal mean ± SE numbers of *B. argentifolii* adults, eggs and nymphs on leaves of DPL 5415 and ST 474 cotton at Maricopa, AZ, 1998

Cultivar	No. adults/leaf-turn	No./cm² leaf disk	
		Eggs	Nymphs
DPL 5415	7.6 ± 0.8b	0.9 ± 0.1b	0.4 ± 0.1b
ST 474	19.4 ± 2.7a	15.0 ± 3.9a	4.1 ± 1.3a

Means of each life stage in a column were significantly different. *F* values were 91.5, 69.9, and 61.3 for adults, eggs, and nymphs, respectively, with df = 2, $P < 0.01$.

Leaf and Leaf Areole Areas. Over all leaf ages, leaf and leaf areole areas for the two cultivars were not significantly different (Table 4). Younger leaves for both cultivars, as expected, had smaller leaf areas than older leaves. The average areole area for leaves on node #1 below the plant terminal was ≈60% of the area for leaves on node #15 (Table 4; Fig. 3). The average vascular bundle areas around and within areoles for leaves from node #7 were 0.113 and 0.111 mm² for ST 474 and DPL 5415, respectively. The difference between cultivars was not significant ($F < 0.1$; df = 1, 4). These results are similar to those from earlier greenhouse studies (1999, A. C. Cohen, personal communication).

Terminal Vein Endings. Over all leaf ages, averages of the numbers of leaf terminal vein endings within areoles for the two cultivars were similar (Table 4). The average numbers of leaf terminal vein endings per square millimeters of leaf area for both cultivars were significantly higher for leaves from nodes #1 (1.23) than older leaves. Differences in numbers of leaf terminal vein endings per square millimeter of leaf area among leaves from node #7 to #20 were not significantly different.

Table 2. Mean ± SE numbers of trichomes on greenhouse grown cotton abaxial leaf surfaces, 1999

Variable	No. trichome/4.5 cm² leaf disk
Cultivar	
ST 474	47.5 ± 5.4a
DPL 5415	2.3 ± 1.1b
Vein classification	
Primary vein	34.0 ± 7.8a
Secondary and tertiary vein	22.2 ± 5.9b
Between veins	18.5 ± 6.9b
Cultivar × vein	
ST 474, Primary vein	61.1 ± 9.2a
Secondary and tertiary vein	44.4 ± 6.0b
Between veins	36.9 ± 11.3c
DPL 5415, Primary veins	6.8 ± 2.8d
Secondary vein	0.0 ± 0.0e
Between vein	0.1 ± 0.1e

Means within a variable not followed by the same letters are significantly different (Student–Newman–Keul's multiple range test, $P = 0.05$). *F* values were 70.0, 15.1, and 32.0 for cultivar, veins and cultivar × veins, respectively, with df = 18, 36, and 54, respectively, $P < 0.01$.

Table 3. Mean ± SE distances from abaxial leaf surface to nearest minor phloem tissue and leaf thicknesses of field grown ST 474 and DPL 5415 cottons, Maricopa, AZ, 1998

Treatment	Distance from abaxial leaf surface to the nearest minor vein phloem tissue, μm	Leaf thickness, μm
Cultivar		
DPL 5415	92.9 ± 3.0a	291.3 ± 9.1a
ST 474	88.0 ± 3.3a	279.5 ± 9.5b
Leaf node		
1	53.8 ± 2.3d	144.0 ± 4.9e
3	81.7 ± 4.0c	258.4 ± 4.6d
5	88.0 ± 3.1bc	283.7 ± 3.8c
7	94.8 ± 3.1bc	313.1 ± 7.9b
10	90.8 ± 4.6bc	303.7 ± 6.2b
15	104.4 ± 4.4b	329.8 ± 6.3b
20	119.8 ± 3.7a	365.1 ± 5.8a
Cultivar × leaf node		
1 DPL 5415	54.3 ± 4.2f	155.7 ± 2.3e
3 DPL 5415	87.5 ± 6.4d	251.0 ± 6.7d
5 DPL 5415	89.7 ± 4.3cde	282.7 ± 6.5c
7 DPL 5415	100.4 ± 4.5bcd	333.6 ± 11.5b
10 DPL 5415	104.8 ± 3.9bcd	324.8 ± 2.9b
15 DPL 5415	101.1 ± 6.3bcd	330.0 ± 8.0b
20 DPL 5415	112.5 ± 5.8b	361.3 ± 6.8a
1 ST 474	53.3 ± 2.4f	132.3 ± 7.6f
3 ST 474	75.8 ± 4.1e	265.8 ± 5.6cd
5 ST 474	86.3 ± 4.6de	284.7 ± 4.4c
7 ST 474	89.2 ± 3.2cde	292.7 ± 4.2c
10 ST 474	76.8 ± 4.5e	282.5 ± 5.2c
15 ST 474	107.7 ± 6.4bc	329.7 ± 10.3b
20 ST 474	127.0 ± 2.9a	368.9 ± 9.8a

Means within a variable in a column not followed by the same letters are significantly different (Student–Newman–Keul's multiple range test, $P = 0.05$). F values for the minor veins were 3.2, 38.9, and 4.4 for cultivar, leaf node and cultivar × leaf node, respectively; and for the leaf thickness were 11.6, 196.5, and 5.4 for cultivar, leaf node and cultivar × leaf node, respectively, with df = 14, 84, and 84, respectively, $P = 0.09$ for minor veins of cultivar and $P < 0.01$ for the other comparisons.

Lysigenous Glands. The average numbers of lysigenous glands over all leaf ages for each cultivar were not significantly different (Table 5). Higher numbers of lysigenous glands per square millimeter of leaf area occurred on leaves from node #1 compared with leaves from nodes #3 to 20. More lysigenous glands were located on adaxial than abaxial leaf surfaces. The average numbers of lysigenous glands per square millimeter of leaf area were significantly higher for leaves from node #1 for both ST 474 and DPL 5415 (1.47–2.27 with an average of 1.85 glands square millimeter for both cultivars). The average numbers of lysigenous glands on leaves from nodes #3 to 20 were variable but not significantly different.

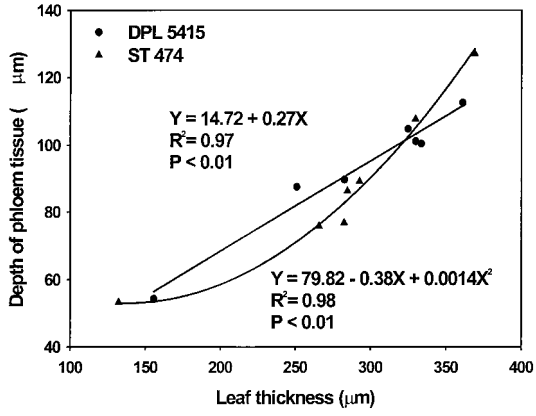

Fig. 2. Relationship between depths of phloem tissue and leaf thicknesses of ST 474 and DPL 5415 cottons.

Table 4. Mean ± SE leaf and leaf areole areas and average numbers of leaf terminal vein endings of different leaf ages of ST 474 and DLP 5415 cottons, Maricopa, AZ, 1998

Treatment	Leaf area, cm^2	Areole area, mm^2	Leaf terminal vein endings, No./mm^2
Cultivar			
ST 474	91.3 ± 10.8a	0.052 ± 0.010a	0.79 ± 0.16a
DPL 5415	97.7 ± 11.2a	0.051 ± 0.007a	0.69 ± 0.09a
Leaf node			
1	11.4 ± 0.9e	0.038 ± 0.005b	1.23 ± 0.08a
3	50.2 ± 3.5d	—	—
5	89.5 ± 4.6c	—	—
7	122.9 ± 6.4b	—	0.70 ± 0.03b
10	134.9 ± 6.9ab	—	0.63 ± 0.05b
15	150.4 ± 12.7a	0.064 ± 0.004a	0.62 ± 0.05b
20	102.3 ± 14.8c		0.50 ± 0.04b

Means within a variable not followed by the same letters are significantly different (Student–Newman–Keul's multiple range test, $P = 0.05$). F values for leaf area were 2.9 and 71.3 for cultivar and leaf node, respectively; for areole area were <0.01 and 65.8 for cultivar and leaf node, respectively; and for leaf terminal vein endings were 6.3, 63, and 25.8 for cultivar and leaf node, respectively. Degrees of freedom were 2 for cultivar of leaf area and areole area; 71 for cultivar of vein endings; and 24, 2, and 68 for leaf node of leaf area, areole area and vein endings, respectively. P were 0.2, none, and 0.01 for leaf area, areole area and leaf terminal vein endings of cultivar, respectively; and <0.01, 0.01, and <0.01 for leaf area, areole area and vein endings of leaf node, respectively. Interactions were not significant.

Laboratory Studies—Leaf Probing Sites of Adults and Settled First and Fourth Instars. Adult females and settled first and fourth instar stylet probing sites on the abaxial leaf surface were found on secondary and tertiary leaf veins and between leaf veins in areoles (Table 6). No feeding sites were found on primary leaf veins or main leaf veins. Adult male and female body lengths averaged 0.95 ± 0.01 mm and 1.20 ± 0.01 mm (mean ± SE, $n = 100$), respectively. Settled first and fourth instar body lengths averaged 0.25 ± 0.002 mm and 0.69 ± 0.005 mm (mean ± SE, $n = 100$), respectively.

Discussion

Greater numbers of *B. argentifolii* adults, eggs, and nymphs occurred on ST 474 than on DPL 5415 (Fig. 1 A–C; Table 1). On average, ST 474 had 20 times more trichomes than DPL 5415. Few trichomes were found on secondary and tertiary veins or between veins on DPL 5415 (Table 2). These results are similar to those reported by other authors (Norman and Sparks 1997, Chu et al. 1998a). There were variations in trichome density on abaxial leaf surface of cotton from one year to the other; however, the density differences be-

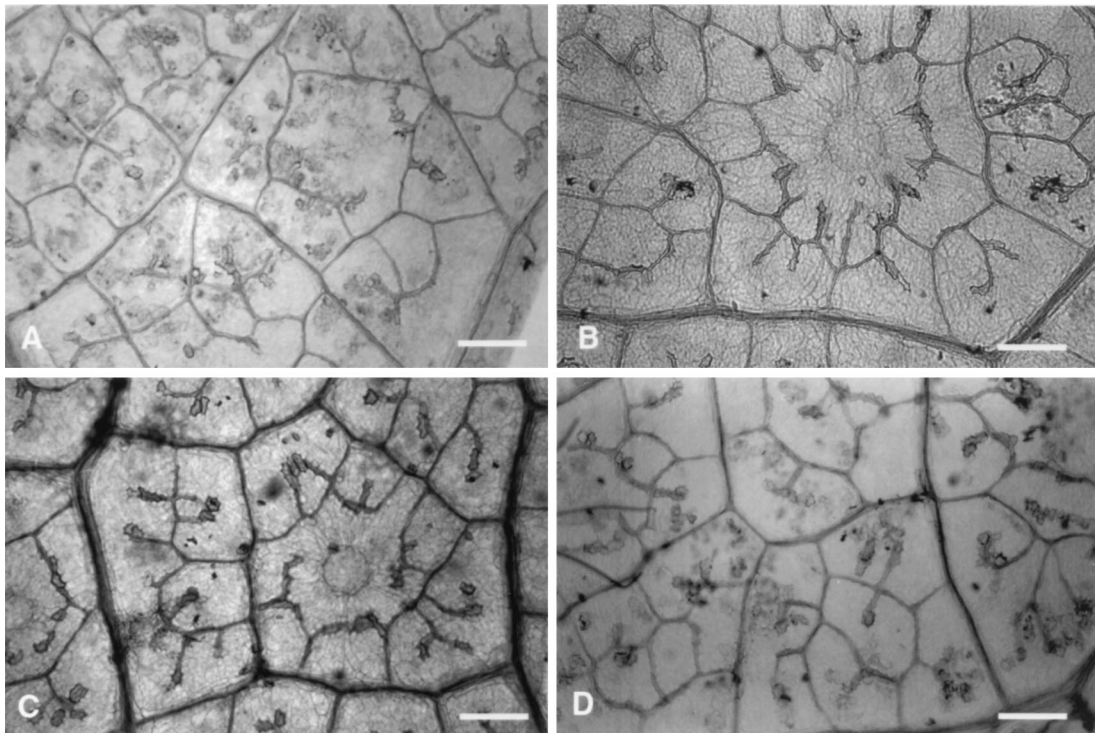

Fig. 3. Electron micrograph of cotton leaf areoles and terminal vein endings from leaves at nodes #1, 7, 15, and 20 (A, B, C, and D, respectively). Note the small leaf areoles in A compared with larger leaf areoles in B and C. Scale bars = 200 μm.

tween the ST 474 and DPL 5415 were consistent each year (Norman and Sparks 1997). A possible explanation for differences in *B. argentifolii* infestation of the two cultivars may be that high leaf trichome densities result in increasing leaf surface boundary layer humidity (Burrage 1971, Wilmer 1986). Subtle variations in boundary layer humidities may provide a better habitat for *B. argentifolii* egg and nymph survival under desert conditions.

Other factors may also be important for *B. argentifolii* feeding and survival. ST 474 had, on average, thinner leaves compared with DPL 5415 (Table 3) and shorter distances from abaxial leaf surface to the nearest vein phloem tissue when leaf thicknesses were less than ≈320 μm (Fig. 2). These relationships presumably provide easier access to phloem tissues for feeding (Chu et al. 1998b, 1999a). The distance to phloem tissues may be particularly important for first instars that have limited time to find feeding sites under desert conditions (Cohen et al. 1998). They probably also have shorter stylet length compared with older nymphs and adults. Our results showed increasing distances of minor vein phloem tissue from the abaxial leaf surfaces with increasing leaf age (Table 3). This may partially explain higher *B. argentifolii* adult densities on leaves near the tops of the plants (Chu et al. 1998c).

Other investigators have suggested that young apical cotton leaves are not selected by *B. tabaci* for oviposition and feeding because the trichome density is too high (Mound 1965, Sippell et al. 1987). Lenteren and Noldus (1990) concluded that moderate as opposed to heavy trichome density habitats were preferred for whitefly colonization. Further study on trichome density on abaxial leaf surfaces may improve our knowledge of the possible relationship to site selection of *B. argentifolii* adults for feeding and oviposition. Our results showed that young leaves are smaller (Table 4), thinner, and have dense networks of veins that are closer to the abaxial leaf surfaces than occurs on older leaves (Table 3). The preference for young leaves by *Bemisia* spp. has been reported in cotton, lettuce, *Lactuca sativa* L. (Byrne and Draeger 1989), and tomatoes (Heinz and Zalom 1995). Young leaves on cotton plants are also yellowish green in contrast to the darker green color of older leaves. In trap studies, we have found that lime green color trap bases with peak light reflection at 520 nm of the light spectrum, attract more *B. argentifolii* adults than yellow (580 nm) or green (510 nm) (Chu et al. 1999b). The difference in light reflectance of younger leaves compared with older leaves may influence attraction of *B. argentifolii* adult females for feeding and oviposition. Also, nutritional value of phloem sap is greater for younger cotton leaves compared with older ones and may be an additional consideration for habitat selection (Tarczynski et al. 1992).

The role of lysigenous glands on the *B. argentifolii* infestation remains unknown, although our results showed that leaves on leaf node #1 had three to four

Table 5. Mean ± SE numbers of lysigenous glands in abaxil and adaxial leaf surfaces of different age of cotton leaves of Stoneville 474 and Deltapine 5415, Maricopa, AZ, 1998

Treatment	No. lysigenous glands/mm^2
Cultivar	
ST 474	0.65 ± 0.09a
DPL 5415	0.60 ± 0.09a
Leaf node	
1	1.85 ± 0.20a
3	0.50 ± 0.04b
5	0.43 ± 0.02b
7	0.44 ± 0.04b
10	0.40 ± 0.02b
15	0.39 ± 0.03b
20	0.38 ± 0.03b
Leaf surface	
Abaxial	0.54 ± 0.07b
Adaxial	0.71 ± 0.10a
Leaf node × leaf surface	
1 Abaxial	1.53 ± 0.22b
1 Adaxial	2.18 ± 0.29a
3 Abaxial	0.46 ± 0.05c
3 Adaxial	0.53 ± 0.06c
5 Abaxial	0.38 ± 0.02c
5 Adaxial	0.48 ± 0.02c
7 Abaxial	0.39 ± 0.04c
7 Adaxial	0.50 ± 0.06c
10 Abaxial	0.37 ± 0.02c
10 Adaxial	0.44 ± 0.03c
15 Abaxial	0.35 ± 0.03c
15 Adaxial	0.42 ± 0.04c
20 Abaxial	0.32 ± 0.02c
20 Adaxial	0.44 ± 0.04c

Means within a variable not followed by the same letters are significantly different (Student–Newman–Keul's multiple range test, $P = 0.05$). F values were 0.5, 28.2, 27.9, and 6.4 for cultivar, leaf node, leaf surface, and leaf node × leaf surface, respectively. Degrees of freedom were 2, 24, 28, and 28 for cultivar, leaf node, leaf surface, and leaf node × leaf surface, respectively. $P = 0.5$ and <0.01 for cultivar and other parameters, respectively. Other interactions were not significant.

times more lysingenous glands than leaves below (Table 6). Future research on lysigenous glands and the small leaf veins within areoles may reveal useful knowledge in *B. argentifolii* feeding behavior.

Our results showing stylet penetration of secondary and tertiary veins and avoidance of main and primary veins appear to confirm our earlier findings that *B. argentifolii* used minor veins for feedings (Cohen et al.1996a, 1996b, 1998) (Table 6). Reasons for the lack of stylet penetration into main or primary veins remain unknown. Stylet penetration also occurred between veins within areole areas where veins smaller than tertiary veins occur but we were not been able to positively identify those smaller veins or *B. argentifolii* feeding with a stereomicroscope. Some of the between vein stylet probing recorded in these studies may actually be probes to leaf terminal vein endings but this will have to be verified in additional studies, because more sophisticated microscopy is essential.

Table 6. Mean ± SE numbers of adult females of *B. argentifolii* and nymph stylet penetration sites on greenhouse grown cotton

Leaf vein	No. stylet probing site/leaf vein type			
	Female adults	Settled 1st instar	4th instar	All nymphs
Main	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Primary	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Secondary	1.3 ± 0.4	2.6 ± 0.4	4.2 ± 0.4	3.4 ± 0.4
Tertiary	0.9 ± 0.4	4.2 ± 2.2	2.2 ± 0.4	3.2 ± 0.6
Between veins	0.4 ± 0.2	3.2 ± 0.6	3.6 ± 0.4	3.4 ± 0.3

Chi-square values were 222.8, 99.5, 89.0, and 94.1 for female adults, settled first instar, fourth instar, and all nymphs, respectively. Degrees of freedom were 4 and $P < 0.01$.

Bemisia argentifolii searching mechanisms for locating phloem tissues within the leaf blade are unknown. The time required for feeding site selection may be an important factor in *B. argentifolii* adult and nymph survival. Trichome location and elongated epidermal cell orientation above veins have been suggested as providing cues for locating minor veins in host leaves (Cohen et al. 1996b). The dense vein network (Fig. 3 A and D), small areole area, high numbers of leaf terminal vein endings (Table 4), and relatively high percentages of vein area in both young and old leaf blades indicate the ready availability of numerous minor veins feeding sites. Considering the body length of adults and settled first instars (≈1.0 and 0.25 mm long, respectively) and the size of leaf areoles (Table 4), a minimal physical movement and random probing may provide easy access to veins from any location of the abaxial leaf surface. Adult females in our studies probed cotton leaf veins 85% of the time compared with 15% of the time probing between veins (Table 6). Also, settled first and fourth instars spent 64 and 68% of their time probing leaf veins, respectively. These results suggest that some other mechanism, in lieu of or in addition to random probing of adults and nymphs, is used for locating leaf veins. Using electronically monitored, tethered female adults, we found that <1 min occurred after release on the leaf surface before stylet probing was initiated; however, 3.2 h lapsed before the first phloem fluid ingestion occurred (unpublished data). This time lapse is in the range observed by G. P. Walker on cotton (1999, University of California, Riverside, CA, personal communication) using the same technique. It appears that stylet probing and location of phloem tissue is an inefficient and poorly understood process. Further work with electronic stylet penetration techniques to refine our knowledge of *Bemisia* phloem finding may provide additional answers to their feeding behavior.

Acknowledgments

We thank Kathy Iverson and Scott Payers for their technical assistance in sectioning of leaf tissues and Patrick Alexander for his assistance in field studies.

References Cited

Anonymous. 1989. MSTAT-C. A microcomputer program for the design, management, and analysis of agronomic research experiments. Michigan State University, East Lansing.

Bell, A. A. 1986. Physiology of secondary products, pp. 597–621. *In* J. R. Mauney and J. McD. Stewart [eds.], Cotton physiology. The Cotton Foundation, Memphis, TN.

Berlinger, M. J. 1986. Host plant resistance to *Bemisia tabaci.* Agric. Ecosys. Environ. 17: 69–82.

Burrage, S. W. 1971. The microclimate at the leaf surface, pp. 91–101. *In* T. F. Preece and C. H. Dickinson [eds.], Biology of leaf surface organisms. Academic, London.

Butler, G. D., Jr., and T. J. Henneberry. 1984. Effect of cotton leaf pubescence on abundance. Southwest. Entomol. 9: 91–94.

Butler, G. D., Jr., F. D. Wilson, and G. Fisher. 1991. Cotton leaf trichome and populations of *Empoasca lybica* and *Bemisia tabaci.* Crop Prot. 10: 461–464.

Byrne, D. N., and E. A. Draeger. 1989. Effect of plant maturity on oviposition and nymphal mortality of *Bemisia tabaci* (Homoptera: Aleyrodidae). Environ. Entomol. 18: 429–432.

Chu, C. C., E. T. Natwick, H. H. Perkins, D. E. Brushwood, T. J. Henneberry, S. J. Castle, Allen C. Cohen, and M. A. Boykin. 1998a. Upland cotton susceptibility to *Bemisia argentifolii* (Homoptera: Aleyrodidae) infestations. J. Cotton Sci. 2: 1–9.

Chu, C. C., E. T. Natwick, A. C. Cohen, G. S. Simmons, D. E. Brushwood, and T. J. Henneberry. 1998b. *Bemisia argentifolii* colonization, vascular bundle depth relationships, lint yield, and nymphal parasitism in selected Deltapine cotton cultivars. Southwest. Entomol. 23: 293–299.

Chu, C. C., T. J. Henneberry, and E. T. Natwick. 1998c. *Bemisia argentifoli* adults caught in CC whitefly traps at different trap heights and trap catch relationships to leaf-turn counts on cotton. Southwestern Entomol. 23: 259–268.

Chu, C. C., A. C. Cohen, E. T. Natwick, G. S. Simmons, and T. J. Henneberry. 1999a. *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype B colonization and leaf morphology relationships in upland cotton. Australian J. Entomol. 38: 127–131.

Chu, C. C., P. J. Pinter, Jr., T. J. Henneberry, K. Umeda, E. T. Natwick, Y. Wei, V. R. Reddy, and M. Shrepatis. 1999b. Catches of silverleaf whiteflies, thrips and leafhoppers with different trap base colored CC traps, p. 9. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge [eds.], Silverleaf whitefly, National Research, Action, and Technology Transfer Plan, 1997–2001: Second Annual Revision of the Second 5-Year Plan. USDA-ARS 1999–01.

Cohen, A. C., T. J. Henneberry, and C. C. Chu. 1996a. Geometric relationships between whitefly feeding behavior and vascular bundle arrangements. Entomol. Exp. Appl. 78: 135–142.

Cohen, A. C., C. C. Chu, T. J. Henneberry, T. Freeman, J. Buckner, and D. Nelson. 1996b. Cotton leaf surface features serve as behavioral cues to silverleaf whiteflies. Southwest. Entomol. 21: 377–385.

Cohen, A. C., C. C. Chu, T. J. Henneberry, T. Freeman, D. Nelson, J. Buckner, D. Margosan, P. Vail, and L. H. Aung. 1998. Feeding biology of the silverleaf whitefly (Homoptera: Aleyrodidae). Chin. J. Entomol. 18: 65–82.

Flint, H. M., and N. J. Parks. 1990. Infestation of germplasm lines and cultivars of cotton in Arizona by whitefly nymphs (Homoptera: Aleyrodidae). J. Entomol. Sci. 25: 223–229.

Heinz, K. M., and F. G. Zalom. 1995. Variation in trichome-based *Bemisia argentifolii* (Homoptera; Aleyrodidae) oviposition on tomato. J. Econ. Entomol. 88: 1494–1502.

Jenkins, J. N. 1994. 1994. Host plant resistance to insects in cotton, pp. 359–372. *In* G. A. Constable and N. W. Forrester [eds.], Challenging The Future: Proceedings, World Cotton Research Conference, Brisbane, Australia, 14–17 February. CSIRO. Melbourne, Australia.

Lenteren, J. C., van, and L.P.J.J. Noldus. 1990. Whitefly-plant relationships: behavioral and ecological aspects, pp. 47–89. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status and management. Intercept. Andover, UK.

McClendon, J. H. 1992. Photographic survey of the occurrence of bundle-sheath extensions in deciduous dicots. Plant Physiol. 99: 1677–1679.

Mound, L. A. 1965. Effect of leaf hair on cotton whitefly populations in the Sudan Gezira. Empire Cotton Growing Rev. 42: 33–40.

Naranjo, S. E., and H. M. Flint. 1994. Spatial distribution of preimaginal *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development of fixed-precision, sequential sampling plans. Environ. Entomol. 23: 254–246.

Naranjo, S. E., and H. M. Flint. 1995. Spatial distribution of adult *Bemisia tabaci* in cotton and development and validation of fixed-precision sequential sampling plans for estimating population density. Environ. Entomol. 24: 261–270.

Natwick, E. T., C. C. Chu, and T. J. Henneberry. 1995. Pima and upland cotton susceptibility to *Bemisia argentifolii* under desert conditions. Southwest. Entomol. 20: 429–438.

Norman, J. W., Jr. and A. N. Sparks, Jr. 1997. Cotton leaf hairs and silverleaf whiteflies in the lower Rio Grande Valley of Texas, pp. 1063–1064. *In* R. Dugger and D. A. Richter [eds.], Proceedings, Beltwide Cotton Production Research Conference. National Cotton Council, Memphis, TN.

Percy, R. G., P. C. Ellsworth, and H. S. Moser. 1997. Silverleaf whitefly resistance screening in Pima cotton genotype, p. 186. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge [eds.], Silverleaf whitefly, 1995 Supplement to the 5-Year National Research and Action Plan. USDA-ARS 1997–02.

Sippell, D. W., O. S. Bindra, and H. Khalifa. 1987. Resistance to whitefly *Bemisia tabaci* in cotton (*Gossypium hirsutum*) in the Sudan. Crop Prot. 6: 171–178.

Tarczynski, M. C., D. N. Byrne, and W. B. Miller. 1992. High performance liquid chromatography analysis of carbohydrates of cotton-phloem sap and honeydew produced by *Bemisia tabaci* feeding on cotton. Plant Physiol. 98: 753–756.

Walker, G. P., and Perring. 1994. Feeding and oviposition behavior of the whiteflies (Homoptera: Aleyrodidae) interpreted from AC electronic feeding monitor waveforms. Ann. Entomol. Soc. Am. 87: 363–374.

Walker, G. P., and A. M. Janssen. 2000. Electronic recording of whitefly (Homoptera: Aleyrodidae) feeding and oviposition behavior. *In* G. P. Walker and E. A. Backus [eds.], Principles and applications of electronic monitoring and other techniques in the study of Homopteran feeding behavior. Thomas Say Publications in Entomology. Entomological Society of America, Lanham, MD.

William, M. R. 1998. Cotton insect losses 1997, pp. 904–925. *In* P. Dugger and D. Richter [eds.], Proceedins, Beltwide Cotton Production Research Conference. National Cotton Council, Memphis, TN.

Wilmer, P. 1986. Microclimate effects on insects at the plant surface, pp. 65–80. *In* B. E. Juniper and T.R.E. Southwood [eds.], Insects and the plant surface. Edward Arnold, London.

Received for publication 7 September 1999; accepted 26 January 2000.